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(54) Title: METHODS AND COMPOSITIONS OF CORRELATING TISSUE KALLIKREIN GENE PROMOTER POLYMORPHISMS WITH ESSENTIAL HYPERTENSION (57) Abstract A method is provided for identifying a human subject as having an increased or decreased risk of developing essential hypertension, comprising determining the presence in the subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with an increased or decreased risk of developing essential hypertension, the presence of the allele identifying the subject as having an increased or decreased risk of developing essential hypertension.		

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**METHODS AND COMPOSITIONS OF CORRELATING TISSUE
KALLIKREIN GENE PROMOTER POLYMORPHISMS WITH
ESSENTIAL HYPERTENSION**

5 This invention was made with government support under the National Institutes of Health grant numbers HL 29397 and HL 44083. The government has certain rights in this invention.

BACKGROUND ART

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FIELD OF THE INVENTION

 The present invention provides methods and compositions for identifying human subjects with an increased risk of having essential hypertension. In particular, this invention relates to the identification and characterization of polymorphisms in the
15 human tissue kallikrein gene promoter correlated with increased or decreased risk of developing essential hypertension.

BACKGROUND ART

 The tissue kallikrein-kinin system has long been implicated in blood pressure
20 regulation by genetic and physiological studies. Urinary excretion of tissue kallikrein is significantly lower in patients with essential hypertension (Elliot et al., 1934; Carretero et al., 1971; Margolius et al., 1971; Margolius et al., 1974) and in genetically hypertensive rats (Carretero et al., 1976; Carretero et al., 1978; Ader et al., 1986; Ader et al., 1987). Total urinary tissue kallikrein excretion was also found to be lower
25 among individuals with parental hypertension than it was in individuals with two normotensive parents (Williams et al., 1987). Transgenic mice over-expressing the human tissue kallikrein gene have been shown to exhibit a hypotensive phenotype (Wang et al., 1994; Song et al., 1995). Further, a study involving 57 Utah pedigrees

indicates that a dominant allele expressed as high total urinary kallikrein excretion may be associated with decreased risk of essential hypertension (Berry et al., 1989).

Identification of DNA polymorphisms provides an efficient way to study the
5 association of genes and diseases by linkage analysis. The human tissue kallikrein gene is located on the long arm of chromosome 19 q13.3-13.4 (Evans et al., 1988). Although a TaqI restriction fragment length polymorphism (RFLP) was found at this locus in a Norwegian population (Berge and Berg, 1991), this genetic marker has only two polymorphisms with unfavored allelic frequencies, making it unsuitable for genetic
10 studies. No significant linkage was found between blood pressure and the kallikrein gene using this marker (Berge and Berg, 1993). A polymorphic microsatellite marker close to the tissue kallikrein gene locus has been identified which is useful for linkage analysis (Richards et al., 1991). However, no previous studies have demonstrated a correlation between specific polymorphisms in the tissue kallikrein gene and blood
15 pressure.

The present invention overcomes this shortcoming by providing methods and compositions for identifying individuals having an increased or decreased risk of developing essential hypertension based on analysis and characterization of a highly
20 polymorphic locus in the promoter region of the human tissue kallikrein.

SUMMARY OF THE INVENTION

The present invention provides a method for identifying a human subject as
25 having an increased risk of developing essential hypertension, comprising determining the presence in the subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with an increased risk of developing essential hypertension, the presence of the allele identifying the subject as having an increased risk of developing essential hypertension.

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Also provided is a method for identifying a human subject as having an increased risk of developing essential hypertension, comprising correlating the presence of a nucleic acid sequence at nucleotides -166 through -98 in a human tissue kallikrein gene with an increased risk of developing essential hypertension; and determining the
5 nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene, whereby a subject having a nucleic acid sequence at nucleotides -166 through -98 in the tissue kallikrein gene correlated with an increased risk of developing essential hypertension is identified as having an increased risk of developing essential hypertension.

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Furthermore, a method of identifying an allele in the promoter region of the human tissue kallikrein gene correlated with an increased risk of developing essential hypertension is also provided, comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the human tissue kallikrein gene from a
15 subject; and correlating the presence of the nucleic acid sequence of the subject with the diagnosis of essential hypertension in the subject, whereby the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene identifies an allele correlated with an increased risk of developing essential hypertension.

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Additionally provided is a method of identifying an allele in the promoter region of a human tissue kallikrein gene correlated with an increased risk of developing essential hypertension comprising: determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue
25 kallikrein gene; cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product less than the

amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the nucleotides at position -166 through -98 of the human tissue kallikrein gene as an
5 allele correlated with an increased risk of developing essential hypertension.

Further provided is a method for identifying a human subject as having an increased risk of developing essential hypertension comprising cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene of the
10 subject into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product less than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the
15 nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a subject as having an increased risk of developing essential hypertension.

In addition, a method for identifying an allele in the promoter region of the
20 human tissue kallikrein gene is provided, comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98.

The present invention further provides an isolated nucleic acid consisting of the promoter region of the human tissue kallikrein gene between positions -1 to -940, the
25 complementary sequence of the isolated nucleic acid, vectors containing the nucleic acid and cells expressing the vector.

The present invention also provides a method of identifying a subject at reduced risk of having essential hypertension, comprising determining the presence in the

subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with a reduced risk of developing essential hypertension, the presence of the allele identifying the subject as having a reduced risk of developing essential hypertension.

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Additionally provided in the present invention is a method for identifying a human subject as having a reduced risk of developing essential hypertension, comprising correlating the presence of a nucleic acid sequence at nucleotides -166 through -98 in a human tissue kallikrein gene with a reduced risk of developing essential hypertension; and determining the nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene, whereby a subject having a nucleic acid sequence at nucleotides -166 through -98 in the tissue kallikrein gene correlated with a reduced risk of developing essential hypertension is identified as having a reduced risk of developing essential hypertension.

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A method is also provided for identifying an allele in the promoter region of the human tissue kallikrein gene correlated with a reduced risk of developing essential hypertension comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the human tissue kallikrein gene from a subject; and correlating the presence of the nucleic acid sequence of the subject with the absence of essential hypertension in the subject, whereby the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene identifies an allele correlated with a reduced risk of developing essential hypertension.

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The present invention further provides a method of identifying an allele in the promoter region of a human tissue kallikrein gene correlated with a reduced risk of developing essential hypertension comprising: determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human

tissue kallikrein gene; cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product greater than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the nucleotides at position -166 through -98 of the human tissue kallikrein gene as an allele correlated with a reduced risk of developing essential hypertension.

Finally provided is a method for identifying a human subject as having a reduced risk of developing essential hypertension comprising cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene of the subject into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product greater than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a subject as having a reduced risk of developing essential hypertension.

Various other objectives and advantages of the present invention will become apparent from the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

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Figure 1. Deletion analysis of the human tissue kallikrein gene promoter. The fusion constructs containing sequential 5'-deletions of allele B and luciferase reporter gene (Luc, open box) are shown schematically. The 5' endpoint of each construct is indicated at the left. Some putative response elements in this region are also shown.

5 The transient transfection assay was done in human embryonic kidney 293 cells. The normalized relative luciferase activity (luciferase/ β -galactosidase units) is calculated by comparison of the luciferase activity with that of the -940/+10 allele B construct as the reference (100%). Mean \pm SEM (n=5 to 12) are shown at right, the number of independent experiments used to calculate these data for each construct is shown in
10 parentheses. “*”, $P < 0.05$.

Figure 2. Promoter activities of various alleles in the polymorphic region of the human tissue kallikrein gene. The -940/+10 deletion constructs containing various alleles and luciferase reporter gene are shown schematically. The transient transfection
15 assay was done in human embryonic kidney 293 cells. The normalized relative luciferase activity (luciferase/ β -galactosidase units) is calculated by comparison of the luciferase activity with that of the -940/+10 allele B construct as the reference (100%). Mean \pm SEM (n=5 to 10) are shown at right, the number of independent experiments used to calculate these data for each construct is shown in parentheses. “*”, $P < 0.05$.

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DETAILED DESCRIPTION OF THE INVENTION

As used in the claims, "a" can include multiples.

25 This invention provides a method for identifying a human subject as having an increased risk of developing essential hypertension, comprising determining the presence in the subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with an increased risk of developing essential hypertension, the presence of the allele identifying the subject as having an increased risk of

developing essential hypertension. The presence of an allele is determined by isolating the promoter region of the subject's tissue kallikrein gene and determining the nucleic acid sequence of the promoter according to standard molecular biology protocols well known in the art as described for example in Sambrook et al. (1989) and as set forth in the Examples provided herein. The nucleic acid sequences of examples of the alleles of the present invention are set forth in Table 1.

An allele in the promoter region of the human tissue kallikrein gene can be identified as correlated with an increased risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular allele in individuals diagnosed with essential hypertension. Furthermore, an allele in the promoter region of the human kallikrein gene can be identified as an allele correlated with an increased risk of developing essential hypertension on the basis of statistical analysis of the incidence of a particular allele in individuals demonstrating a decrease in the amount of urinary excretion of tissue kallikrein relative to the average amount of urinary excretion in a large sample of normotensive individuals. For example, the amount of tissue kallikrein excreted in the urine by subjects who have specific alleles can be determined according to methods well known in the art as described herein as well as in, for example, Shimamoto et al (1984). The amount of tissue kallikrein excreted in the urine by subjects diagnosed with essential hypertension can be determined as described herein. The data provided for these parameters can be used to establish a correlation between a specific allele in the promoter region of the human tissue kallikrein gene (genotype) and an increased risk of developing essential hypertension (phenotype).

In addition, an allele in the promoter region of the human tissue kallikrein gene can be identified as an allele correlated with an increased risk of developing essential hypertension through molecular biology protocols which measure the activity of the promoter of the human tissue kallikrein gene with various alleles. For example, as described in the Examples section herein, different alleles can be identified and cloned

into vectors and positioned to control the transcription of a reporter gene such as, for example, luciferase, although any reporter gene known in the art would be suitable. The amount of reporter gene product produced can be quantitated as described herein to measure the activity of a particular allele in the promoter region of the human tissue kallikrein gene. The activity of the promoter can also be determined by other protocols available in the art for measuring gene activity, including, for example, the chloramphenicol acetyltransferase assay (Sambrook et al., 1984) and the green fluorescent protein assay (GIBCO BRL Products and Reference Guide, Life Technologies, Gaithersburg, MD, 1997).

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A promoter with a particular allele having less activity than a promoter with the nucleotide sequence of allele A (SEQ ID NO:14) or the nucleotide sequence of allele B (SEQ ID NO:15) identifies an allele correlated with an increased risk of developing essential hypertension. For example, an allele correlated with an increased risk of developing essential hypertension can be, but is not limited to, allele D (SEQ ID NO:17) or allele H (SEQ ID NO:20). Using data similar to that obtained for alleles D and H, other alleles can also be correlated with an increased risk of developing essential hypertension.

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The amount of activity which is less than the amount of activity of a promoter containing the reference nucleotide sequence of allele A (SEQ ID NO:14) or the reference nucleotide sequence of allele B (SEQ ID NO:15) can be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50% less or greater than 50% less than the reference sequence activity. The amount of activity which is less than the reference sequence activity can also be in a range of, for example, between 1% and 10% less, between 11% and 49% less and 50% less or greater than 50% less than the reference sequence activity.

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Essential hypertension is considered to be a quantitative disorder and is generally characterized in terms of marginal, moderate or severe hypertension. For example, marginal essential hypertension can be generally defined by a systolic blood pressure value greater than 120 mmHg, up to about 140 mmHg and diastolic blood pressure value greater than 90, up to about 100 mmHg. Moderate essential hypertension can be generally defined by a systolic blood pressure greater than 140 mmHg, up to about 180 mmHg and a diastolic blood pressure value greater than 100 mmHg, up to about 120 mmHg. Severe essential hypertension can be generally defined by a systolic blood pressure value greater than 180 mmHg and a diastolic blood pressure value greater than 120 mmHg. These values do not define the absolute limits for the classification of essential hypertension as marginal, moderate or severe and can vary up to ± 5 mmHg for each value. It is also contemplated in the present invention that a positive correlation can exist between the percent decrease in promoter activity as compared to alleles A and B and the classification of the hypertension as marginal, moderate or severe.

The data provided herein describe promoter activity in human kidney 293 cells (ATCC CRL 1573). However, the present invention contemplates the use of any human cells which express the tissue kallikrein gene, such as any human pancreatic, kidney, prostate and salivary gland cell lines. Cells from other species which express the tissue kallikrein gene (e.g., mouse, rat) can be also be used in the present invention. For example, the cells of the present invention can be P5 human proximal kidney tubule cells (Medical University of South Carolina, Department of Biochemistry and Molecular Biology), LNCaP human prostate cells (ATCC CRL 1740), HSG human salivary gland cells (National Institute of Dental Research) and the like.

Also provided in the present invention is a method for identifying a human subject as having an increased risk of developing essential hypertension, comprising correlating the presence of a nucleic acid sequence at nucleotides -166 through -98

(Evans et al. 1989) in a human tissue kallikrein gene with an increased risk of developing essential hypertension; and determining the nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene, whereby a subject having a nucleic acid sequence at nucleotides -166 through -98 in the tissue kallikrein gene correlated with an increased risk of developing essential hypertension is identified as having an increased risk of developing essential hypertension.

The nucleic acid sequence of nucleotides -166 through -98 of a subject's tissue kallikrein gene can be determined, for example, as described herein, according to standard nucleic acid sequencing protocols which are well known in the art. The nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene can be correlated with an increased risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular nucleic acid sequence of nucleotides -166 through -98 in individuals diagnosed with essential hypertension. Furthermore, a nucleic acid sequence of nucleotides -166 through -98 can be correlated with an increased risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular nucleic acid sequence of nucleotides -166 through -98 and an increase of the amount of urinary excretion of tissue kallikrein relative to normotensive subjects. For example, the amount of tissue kallikrein excreted in the urine by subjects who have specific nucleic acid sequences at nucleotides -166 through -98 can be determined according to methods well known in the art as described herein as well as in Shimamoto et al. (1984) and the amount of tissue kallikrein excreted in the urine by subjects diagnosed with essential hypertension can be determined as described herein. These data can be used to establish a correlation between a specific nucleic acid sequence of the nucleotides -166 through -98 in the human tissue kallikrein gene and an increased risk of developing essential hypertension.

In addition, a particular nucleic acid sequence of nucleotides -166 through -98 of the human tissue kallikrein gene can be correlated with an increased risk of developing essential hypertension as determined through molecular biology protocols which measure the activity of the promoter of the human tissue kallikrein gene with various nucleic acid sequences at nucleotides -166 through -98 of the tissue kallikrein gene. For example, as described in the Examples section herein, the promoter region of the tissue kallikrein gene with various nucleic acid sequences at nucleotides -166 through -98 can be cloned into vectors and positioned to control the transcription of a reporter gene such as, for example, luciferase. The amount of reporter gene product produced can be quantitated as described herein to measure the activity of a promoter of the human tissue kallikrein gene having a particular nucleic acid sequence at nucleotides -166 through -98.

A promoter containing a nucleic acid sequence at nucleotides -166 through -98 having less activity than a promoter containing the nucleotide sequence of allele A (SEQ ID NO:14) or the nucleotide sequence of allele B (SEQ ID NO:15) identifies a nucleic acid sequence at nucleotides -166 through -98 correlated with an increased risk of developing essential hypertension. As described above, the percent decrease in activity demonstrated by a particular nucleic acid sequence at nucleotides -166 through -98 is directly correlated with an increased risk of developing a particular degree (i.e., marginal, moderate or severe) of essential hypertension. For example, as set forth above, nucleic acid sequence at nucleotides -166 through -98 correlated with an increased risk of developing severe essential hypertension can be, but is not limited to, the nucleic acid sequences at nucleotides -166 through -98 comprising allele D (SEQ ID NO:17) or comprising allele H (SEQ ID NO:20).

In addition, a method of identifying an allele in the promoter region of the human tissue kallikrein gene correlated with an increased risk of developing essential hypertension is provided. The method comprises determining the nucleic acid sequence

of the nucleotides at positions -166 through -98 in the human tissue kallikrein gene from a subject; and correlating the presence of the nucleic acid sequence of the nucleotides at positions -166 through -98 of the subject with the presence of essential hypertension in the subject, whereby the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene identifies an allele correlated with an increased risk of developing essential hypertension. The methods for correlating the presence of a particular nucleic acid sequence at nucleotides -98 through -166 are as described herein.

10 An additional method of identifying an allele in the promoter region of a human tissue kallikrein gene correlated with an increased risk of developing essential hypertension is also provided. The method comprises determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene; cloning the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product less than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing a nucleic acid sequence of the nucleotides at positions -166 through -98 comprising the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the nucleotides at position -166 through -98 of the human tissue kallikrein gene as an allele correlated with an increased risk of developing essential hypertension. As stated above, a positive correlation can exist between the percent decrease in promoter activity as compared to alleles A and B and the classification of the hypertension as marginal, moderate or severe.

The nucleic acid sequence of nucleotides -98 through -166 can be obtained according to standard nucleic acid sequencing protocols standard in the art, as described herein, for example. Cloning and expression of a reporter gene under the control of a tissue kallikrein gene promoter having various nucleic acid sequences at nucleotides -

5 98 through -166 can also be carried out, for example, as described in the Examples herein, using molecular biology protocols for cloning and expression as are well known in the art. Measuring the amount of reporter gene product can be carried out by a variety of methods, depending on the nature of the reporter gene product. A protocol for quantitating the amount of luciferase as a reporter gene product is described in the

10 Examples herein. Other reporter genes and methods for detection of their gene products, such as the chlormaphenicol acetyltransferase assay and the green fluorescent protein assay, would be well known to the artisan and can also be employed in the present invention to measure the activity of a tissue kallikrein gene promoter having a particular nucleic acid sequence at nucleotides -98 through -166.

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Furthermore, the present invention provides a method for identifying a human subject as having an increased risk of developing essential hypertension comprising cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene of the subject into an expression vector having a reporter gene;

20 expressing the reporter gene to produce a gene product under the direction of the promoter region of the subject's human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product which is less than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele

25 A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a subject as having an increased risk of developing essential hypertension. As stated herein, a positive correlation can exist between the percent decrease in activity relative to the activity of promoters containing alleles A or B and the classification of the hypertension as mild, moderate or severe.

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In this embodiment, the exact nucleic acid sequence of the subject need not be known. The promoter region of the subject's kallikrein gene can be cloned into a vector containing a reporter gene according to standard protocols as described herein. The reporter gene product can be produced and quantitated as described herein to
5 determine the amount of activity of the subject's kallikrein gene promoter.

The present invention also provides a method for identifying an allele in the promoter region of the human tissue kallikrein gene, comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98 of the human
10 tissue kallikrein gene. The kallikrein gene can be isolated and the promoter region sequenced according to well defined molecular biology protocols, for example, as described herein. The present invention provides alleles A, B, C, D, E, F, G H, I, J, K, M, P and R as described in Table 1. These 14 alleles were detected in a GC-rich region between -133 bp and -121 bp upstream to the transcription initiation site in Caucasians,
15 African-Americans and Asians. Thus, the present invention further provides isolated nucleic acids consisting of the nucleotide sequences of SEQ ID NO:14 (allele A), SEQ ID NO:15 (allele B), SEQ ID NO:16 (allele C), SEQ ID NO:17 (allele D), SEQ ID NO:18 (allele E), SEQ ID NO:19 (allele F), SEQ ID NO:20 (allele H), SEQ ID NO:21 (allele I), SEQ ID NO:22 (allele K), SEQ ID NO:23 (allele P), SEQ ID NO:24 (allele
20 G), SEQ ID NO:25 (allele J), SEQ ID NO:26 (allele M) and SEQ ID NO:27 (allele R). By "isolated" is meant separated from other nucleic acids found in the naturally occurring organism. Additional alleles can be identified according to the protocols set forth herein.

25 The present invention further provides an isolated nucleic acid consisting of the promoter region of the human tissue kallikrein gene between nucleotides -1 to -940 (Evans et al. 1988). The promoter region of the human tissue kallikrein gene has been defined as that region of the gene upstream of the coding region which confers activity to the gene. By the methods described herein for measuring promoter activity, a region

of the tissue kallikrein gene which imparts activity to the gene resides in the nucleic acid sequence between nucleotides -1 to -940. Examples of these nucleic acids include those comprising alleles A, B, C, D, E, F, H, I, K or P.

5 In addition, an isolated nucleic acid comprising the nucleic acid sequence of the primer designated hKPMA (5'-ATTCTGGGGATCGGAGGG-3') (SEQ ID NO:9) and an isolated nucleic acid comprising the nucleic acid of the primer designated hKPMB (5'-TCTGGGGATCGGAGGC-3') (SEQ ID NO:10), are provided. The isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO:9 or SEQ ID NO:10 can range
10 in size from 16 (SEQ ID NO:10) or 18 nucleotides (SEQ ID NO:9), respectively, to an upper limit of about 940 nucleotides, including any number of nucleotides in that range. By "isolated" is meant separated from other nucleic acids found in the naturally occurring organism. These nucleic acids can be used as forward primers in an allele specific amplification (ASA) method to identify the presence or absence of the two
15 most frequently occurring alleles, A or B, in a subject as described herein. These primer sequences are specific for the A and B alleles, respectively and can be employed in a PCR protocol optimized as described in the Examples herein to distinguish alleles A and B from other alleles on the basis of a variation at a single nucleotide in the promoter region of the tissue kallikrein gene.

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In another embodiment, the present invention provides a method of identifying a subject as having a reduced risk of developing essential hypertension, comprising determining the presence in the subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with a reduced risk of developing essential
25 hypertension, the presence of the allele identifying the subject as having a reduced risk of developing essential hypertension. The presence of an allele correlated with a reduced risk of developing essential hypertension is determined by isolating the promoter region of the subject's tissue kallikrein gene and determining the nucleic acid sequence of the promoter according to standard molecular biology protocols well

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known in the art as described for example in Sambrook et al. (1989) and as set forth in the Examples provided herein.

An allele in the promoter region of the human tissue kallikrein gene can be
5 identified as correlated with a reduced risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular allele in individuals without essential hypertension according to the 2-tailed t test, which is well known in the art. Furthermore, an allele in the promoter region of the human kallikrein gene can be identified as an allele correlated with a reduced risk of developing essential
10 hypertension on the basis of statistical analyses of the incidence of a particular allele in individuals demonstrating an increased amount of urinary excretion of tissue kallikrein, relative to the average amount of urinary excretion of tissue kallikrein as determined for a large number of normotensive individuals. For example, the amount of tissue kallikrein excreted in the urine by subjects who have specific alleles can be determined
15 according to method well known in the art as described herein as well as in Shimamoto et al. (1984) and the amount of tissue kallikrein excreted in the urine by subjects who do not have essential hypertension can be determined as described herein. These data can be used to establish a correlation between a specific allele in the promoter region of the human tissue kallikrein gene and a reduced risk of developing essential
20 hypertension. For example, allele K has been found to be correlated with an increased amount of urinary tissue kallikrein excretion relative to average amounts excreted by normotensive individuals. Therefore, an allele of the present invention correlated with a reduced risk of developing essential hypertension can be, but is not limited to, allele K (SEQ ID NO:22).

25

In addition, an allele in the promoter region of the human tissue kallikrein gene can be identified as an allele correlated with a reduced risk of developing essential hypertension as determined through molecular biology protocols which measure the activity of the promoter of the human tissue kallikrein gene with various alleles. For

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example, as described in the Examples section herein, different alleles can be cloned into vectors and positioned to control the transcription of a reporter gene such as, for example, luciferase. The amount of reporter gene product produced can be quantitated as described herein to measure the activity of a particular allele in the promoter region of the human tissue kallikrein gene. A promoter containing an allele having more activity than a promoter containing allele A (SEQ ID NO:14) or a promoter containing allele B (SEQ ID NO:15) identifies an allele correlated with a reduced risk of developing essential hypertension.

10 Additionally, the present invention provides a method for identifying a human subject as having a reduced risk of developing essential hypertension comprising correlating the presence of a nucleic acid sequence at nucleotides -166 through -98 in a human tissue kallikrein gene with a reduced risk of developing essential hypertension; and determining the nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene, whereby a subject having a nucleic acid sequence at nucleotides -166 through -98 in the tissue kallikrein gene correlated with a reduced risk of developing essential hypertension is identified as having a reduced risk of developing essential hypertension.

20 The nucleic acid sequence of nucleotides -166 through -98 of a subject's tissue kallikrein gene can be determined, for example as described herein, according to standard nucleic acid sequencing protocols which are well known in the art. The nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene can be correlated with a reduced risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular nucleic acid sequence of nucleotides -166 through -98 in individuals who are normotensive, i.e., who do not have essential hypertension. Furthermore, a nucleic acid sequence of nucleotides -166 through -98 can be correlated with a reduced risk of developing essential hypertension on the basis of statistical analyses of the incidence of a particular nucleic acid sequence

of nucleotides -166 through -98 with an increase in the amount of urinary excretion of tissue kallikrein in a subject relative to the average amount of urinary tissue kallikrein excreted by normotensive individuals. For example, the amount of tissue kallikrein excreted in the urine by subjects who have specific nucleic acid sequences at
5 nucleotides -166 through -98 can be determined according to methods well known in the art as described herein as well as in Shimamoto et al. (1984) and the amount of tissue kallikrein excreted in the urine by subjects who are normotensive can be determined as described herein and the data provided can be used to establish a correlation between a specific nucleic acid sequence of the nucleotides -166 through -
10 98 in the human tissue kallikrein gene and a reduced risk of developing essential hypertension.

In addition, a particular nucleic acid sequence of nucleotides -166 through -98 of the human tissue kallikrein gene can be correlated with a reduced risk of developing
15 essential hypertension as determined through molecular biology protocols which measure the activity of the promoter of the human tissue kallikrein gene with various nucleic acid sequences at nucleotides -166 through -98 of the tissue kallikrein gene. For example, as described in the Examples section herein, the promoter region of the tissue kallikrein gene with various nucleic acid sequences at nucleotides -166 through -
20 98 can be cloned into vectors and positioned to control the transcription of a reporter gene such as, for example, luciferase. The amount of reporter gene product produced can be quantitated as described herein to measure the activity of a promoter of the human tissue kallikrein gene having a particular nucleic acid sequence at nucleotides -
25 166 through -98. A promoter containing a nucleic acid sequence at nucleotides -166 through -98 having more activity than a promoter containing the nucleotide sequence of allele A (SEQ ID NO:14) or the nucleotide sequence of allele B (SEQ ID NO:15) identifies a nucleic acid sequence at nucleotides -166 through -98 correlated with a reduced risk of developing essential hypertension.

A method of identifying an allele in the promoter region of the human tissue kallikrein gene correlated with a reduced risk of developing essential hypertension is provided herein as well, comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the human tissue kallikrein gene from a subject; and correlating the presence of the nucleic acid sequence of the subject with the lack of essential hypertension in the subject, whereby the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene identifies an allele correlated with a reduced risk of developing essential hypertension. The methods for correlating the presence of a particular nucleic acid sequence at nucleotides -98 through -166 with a reduced risk of developing essential hypertension are as described herein.

Also provided is a method of identifying an allele in the promoter region of a human tissue kallikrein gene correlated with a reduced risk of developing essential hypertension comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene; cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product more than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing a nucleic acid sequence of the nucleotides at positions -166 through -98 comprising the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the nucleotides at position -166 through -98 of the human tissue kallikrein gene as correlated with a reduced risk of developing essential hypertension.

The nucleic acid sequence of nucleotides -98 through -166 can be obtained according to standard nucleic acid sequencing protocols standard in the art, as described herein, for example. Cloning and expression of a reporter gene under the control of a tissue kallikrein gene promoter having various nucleic acid sequences at nucleotides -
5 98 through -166 can also be carried out, for example, as described in the Examples herein, using molecular biology protocols for cloning and expression as are well known in the art. Measuring the amount of reporter gene product can be carried out by a variety of methods, depending on the nature of the reporter gene product. A protocol for quantitating the amount of luciferase as a reporter gene product is described in the
10 Examples herein. Other reporter genes and methods for detection of their gene products would be well known to the artisan and can also be employed in the present invention to measure the activity of a tissue kallikrein gene promoter having a particular nucleic acid sequence at nucleotides -98 through -166.

15 Furthermore, the present invention provides a method for identifying a human subject as having a reduced risk of developing essential hypertension comprising cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene of the subject into an expression vector having a reporter gene; expressing the reporter gene to produce a gene product under the direction of the
20 promoter region of the subject's human tissue kallikrein gene; and determining the amount of reporter gene product produced, an amount of reporter gene product greater than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) or the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a subject as
25 having a reduced risk of developing essential hypertension.

In this embodiment, the exact nucleic acid sequence of the subject need not be known. The promoter region of the subject's kallikrein gene can be cloned into a vector containing a reporter gene according to standard protocols as described herein.

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The reporter gene product can be produced and quantitated as described herein to determine the amount of activity of the subject's kallikrein gene promoter. A subject determined to have a promoter with greater activity than promoters containing the nucleotide sequences of alleles A and B is identified as a subject having a reduced risk of developing essential hypertension.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

10

EXAMPLES

Population and preparation of genomic DNA. DNA from 108 unrelated subjects including 57 Caucasians, 33 African-Americans and 18 Asians were used for this study. Human genomic DNA was prepared from peripheral blood leukocytes as described previously (Bell et al., 1981).

Amplification of the 5' flanking region of tissue kallikrein gene and direct DNA sequencing. A 996 bp fragment of the 5' flanking region of the human tissue kallikrein gene was amplified by PCR using oligonucleotide primers hKPM1 (5'-CTGCAGGAATCTAGTTC-3') (SEQ ID NO:1) and HUKATG (5'-CAGAACCAGGAACCACAT-3') (SEQ ID NO:2) derived from the hKLK1 sequence (Evans et al., 1988). PCR was performed in a 50 µl reaction mixture containing 5 µl of 10x PCR buffer, 5 µl of 2 mM dNTP, 50 pmoles of each primer, 2.5 U of Taq DNA polymerase and 300 ng of genomic DNA. Thirty cycles of PCR were performed in a thermal cycler with a denaturing phase of 1 min at 94°C, annealing phase of 2 min at 50°C and extension phase of 3 min at 72°C. The resulting PCR products were purified by STE Select-D G50 columns (5Prime--3Prime). An aliquot (5 µl) of the purified PCR product was used for DNA sequencing using the primers

hKPM2 (5'-ATCTGGAGGCACTAGGA-3') (SEQ ID NO:3), hKPM3
(5'-TCCTAGTGCCTCCAGAT-3') (SEQ ID NO:4), hKPM4
(5'-GTACCAGGGTCACTGAT-3') (SEQ ID NO:5), hKPM5
(5'-ATCAGTGACCCTGGTAC-3') (SEQ ID NO:6), hKPM6
5 (5'-GAATGCGTCCAGCGTGATCC-3') (SEQ ID NO:7) and hKPM7
(5'-AGCCCTTATCCCCTCAGAGC-3') (SEQ ID NO:8). Sequencing was performed
with a double-stranded DNA cycle sequencing system (BRL) following the protocol
recommended by the manufacturer. The sequence of the polymorphic region was
verified by sequencing the complementary strands. PCR and sequencing were repeated
10 at least twice to eliminate the possibility of introducing mutations during PCR
amplification. Oligonucleotides were synthesized by the phosphoramidite method
using the ABI (Applied Biosystems) Model 380B DNA synthesizer.

Single-stranded conformation polymorphism analysis (SSCP). The primers used
15 for SSCP were hKPM6 and hKPM7. The 5' primer hKPM6 (20 pmol) was end-labeled
with [γ -³²P] (40 μ Ci) ATP and 10 U of T4 DNA kinase (BRL) in a total volume of 20
 μ l at 37°C for 30 min. The labeled hKPM6 was purified with STE Select-D G25
column (5Prime--3Prime). PCR was performed in a 25 μ l reaction mixture containing
25 pmol of unlabeled primers, 1.25 pmol of labeled hKPM6, 0.2 mM dNTP, 2.5 μ l of
20 10x PCR buffer, 100 ng genomic DNA and 2.5 U of Taq DNA polymerase. Thirty
cycles of PCR (94°C/1 min, 60°C/2 min, 72°C/3 min) were performed. PCR reaction
mixtures were combined with 25 μ l of stop solution (95% formamide, 20 mM EDTA,
0.05% bromophenol blue and 0.05% xylene cyanol), heated at 95°C for 5 min and put
on ice. Samples of 1 μ l each were applied to a 6% non-denaturing polyacrylamide gel
25 containing 90 mM Tris-borate, 4 mM EDTA, pH 8.3 and 10% glycerol.
Electrophoresis was performed at 31 W (approximately 25 V/cm) for 3 hours at room
temperature with cooling using a fan. The gel was dried on filter paper and exposed to
Kodak X-Omat AR film at -80°C for 15 hours.

Allele-specific amplification (ASA). A 180 bp fragment containing the polymorphic region was amplified using hKPM6 and hKPM7 primers. The reaction was performed with 25 ng of genomic DNA in a 25 µl reaction mixture containing 25 pmol of primers, 0.2 mM dNTP, 2.5 µl of 10x PCR buffer and 2.5 U of Taq DNA polymerase using the same PCR program as for SSCP. The PCR products were diluted 1:100 with water. Two allele-specific primers were used in this experiment, hKPM A (5'-ATTCTGGGGATCGGAGGG-3') (SEQ ID NO:9) and hKPM B (5'-TCTGGGGATCGGAGGC-3') (SEQ ID NO:10). PCR was performed in a 25 µl total volume containing 20 pmol of primers (hKPM7 and either hKPM A or hKPM B), 0.2 mM dNTP, 2.5 µl of 10x PCR buffer, 2.5 U of Taq DNA polymerase and 0.2 µl of diluted PCR products from the first round amplification. Thermal cycling pattern was 94°C/1 min, 66°C/40 sec and 72°C/20 sec. After 32 cycles of PCR, the products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide and visualized by ultraviolet illumination.

15

Sequencing gel-based PCR analysis. The primers used in this experiment were hKPM8 (5'-GTGATCCAGGGCCTGCA-3') (SEQ ID NO:11) and hKPM9 (5'-CTTCCCCTGTTGCCTGG-3') (SEQ ID NO:12). The 5' primer, hKPM9 (20 pmol), was end-labeled and purified as described above. PCR was performed using 20 pmol each of unlabeled hKPM8 and hKPM9, 1 pmol of end-labeled hKPM9, 0.2 mM dNTP, 2.5 µl of 10x PCR buffer, 2.5 U of Taq DNA polymerase and 1 µl of the first round PCR product from the ASA. Samples were amplified for 30 cycles, each consisting of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C. The PCR products were mixed with 12.5 µl of stop solution and denatured at 95°C for 5 min. Samples of 1 µl each were loaded onto 8% sequencing gels and electrophoresed at 2000 V (approximately 50 V/cm) for 2.5 hours at room temperature. A sequence reaction of a known gene was used as a size marker in the electrophoresis on the polyacrylamide gel. The gel was dried on filter paper and exposed to Kodak X-Omat AR film at -80°C for 5 hours.

25

Plasmid constructions. Human genomic DNA (25 ng) of the individuals with the different alleles was used as template to amplify promoter sequences spanning nucleotides -940 to +10 from the transcription initiation site of the human tissue kallikrein gene. The PCR was performed with the upstream primer hKPM1 and the downstream primer hKPM15 (5'-TGGAGGAACTGGGGAAC-3') (SEQ ID NO:13). The PCR products were gel-purified, filled-in with Klenow fragment of DNA polymerase I (BRL) and phosphorylated with T4 kinase (BRL). These DNA fragments were cloned into the XhoI site of the vector pGL2-Basic (Promega) which carries the luciferase structural gene. The fidelity of all constructs and insert orientation were determined by restriction enzyme mapping and nucleotide sequencing. The plasmid pCH110, which contains a functional LacZ gene under the direction of the SV40 early promoter, was purchased from Pharmacia. The plasmids used in the transfection studies were prepared with a DNA purification kit (Qiagen).

Cell culture. The human embryonic kidney cell line 293 (ATCC CRL 1573) was maintained in Dulbecco's modified Eagle's medium (D-MEM) (BRL) supplemented with 10% fetal calf serum. These cells were passaged 1:10 weekly at 37°C in a 5% CO₂ incubator.

Transient transfection assays. Human 293 cells were co-transfected with 20 µg of hKLK1 promoter-luciferase reporter gene plasmids and 10 µg of plasmid pCH110. Electroporation was carried out with 6 x 10⁶ cells in 200 µl of D-MEM medium using 0.2-cm gap cuvettes and an electro-cell manipulator-600 (BTX). One shock was administered at 125 V and a capacitance of 800 microfarads. The cells were transferred to 6-well plates following the electroporation and the medium was changed in 24 hours. The cells were harvested 48 hours after replacement with fresh medium.

Luciferase and β-galactosidase assays. The cells were lysed in 110 µl of 1x Reporter Lysis Buffer (Promega) by incubation at room temperature for 15 minutes.

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Luciferase activity was assessed using the Promega Luciferase Assay System and a scintillation counter following the protocol recommended by the manufacturer. To correct for variations in transfection efficiency, β -galactosidase activity was determined. Briefly, 30 μ l of cell extract was added to 30 μ l of assay buffer containing
5 1.33 mg/ml *o*-nitrophenyl- β -D-galactopyranoside, 2 mM MgCl_2 , 100 mM β -mercaptoethanol, 120 mM Na_2HPO_4 and 80 mM NaH_2PO_4 (pH 7.3). The reactions were incubated at 37°C for up to 3 hours and terminated by the addition of 100 μ l of 1 M Na_2CO_3 . The optical density of the reaction was read at 420 nm with a spectrophotometer. The normalized luciferase/ β -galactosidase activity for each plasmid
10 was expressed as a percent of the activity recorded for the -940/+10 type B construct.

Statistical analysis. Allelic frequencies were calculated from the observed numbers of each allele and the total numbers of chromosomes examined. Based on the allelic frequencies, the observed genotypic distributions in this polymorphic region
15 were tested to determine if they conform to Hardy-Weinberg equilibrium expectations based on the method described by Khoury et al. (1993). In the promoter analysis, the data for each construct were expressed as mean \pm SEM. Comparison of the promoter activities between various constructs was made by the Students' *t*-test. Differences were considered significant at a value of $P < 0.05$.

20

Identification of polymorphism in the 5' flanking region of human tissue kallikrein gene. The 5' flanking region of the human tissue kallikrein gene was searched for polymorphisms in 108 unrelated subjects, consisting of 57 Caucasians, 33 African-Americans and 18 Asians. A genomic fragment of this region between +58 bp
25 and -940 bp, was amplified by PCR and subjected to direct DNA sequencing. The fidelity of PCR amplification was ensured by sequencing two batches of the PCR products. Extensive variation was noticed in a GC-rich region located between -133 bp and -121 bp with respect to the transcription initiation site. Ten alleles were identified at this locus. These alleles vary in length from 12 bp to 15 bp and/or in nucleotide

sequence by single-base transversion (Table 1). The greatest variability was found in the African-American population in which all of the alleles were detected. Six alleles (A, B, E, H, I and K) were observed in Caucasian subjects, including four length variations. Four alleles (A, B, H and K) were found in Asians.

5

Allelic frequency distribution and Test of Hardy-Weinberg Equilibrium. Table 2 summarizes the genotype frequencies of alleles in Caucasians, African-Americans and Asians. Alleles A and B have the highest prevalence in these subjects. Alleles H and K are also present with high frequencies in these three ethnic populations. These results show that the shared alleles between the three populations are the most frequent ones. Table 3 summarizes the observed and expected numbers of heterozygotes and homozygotes for each population. The calculation is based on the observed allelic frequencies. The results show that the polymorphisms exhibit Hardy-Weinberg equilibrium in the samples examined.

10
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SSCP analysis. SSCP analysis was performed to determine its efficiency in identifying the alleles in this polymorphic region. Primer hKPM6 was labeled at the 5'-end and included in PCR amplification of a 180 bp fragment that encompassed the polymorphic region. The products were denatured and analyzed on non-denaturing polyacrylamide gels. Eleven samples, including 10 alleles in the form of either homozygotes or heterozygotes, were subjected to SSCP analysis. The SSCP band patterns were found to be reproducible under identical experimental conditions. Although the band mobilities were quite different between some genotypes such as AK heterozygote and II homozygote, other genotypes showed the same or very similar band mobilities in SSCP, such as BB and DD. Therefore, SSCP confirmed the existence of polymorphisms in this region, but does not appear to be suitable for genotyping of this polymorphic region due to the potential ambiguities.

20
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Allele-specific amplification. ASA is a general method which can be optimized to detect all possible single-base mutations. In this polymorphic region, alleles A and B have the highest frequencies in all populations examined. They have a single-base difference in nucleotide sequence at position -128. It would be useful if these alleles could be rapidly genotyped. Two allele-specific upstream primers (hKPMA and hKPMB), differing in their 3' terminal nucleotide, were designed for alleles A and B. Both alleles accepted the same downstream primer for PCR. The PCR conditions were optimized to ensure that the mismatched primer would not function as the perfectly matched primer. A nested PCR protocol generated a 116 bp fragment. Each sample was examined using the two different primer sets in parallel and the results were consistent with those obtained by direct DNA sequencing.

Direct PCR analysis. This method was designed to facilitate the rapid assignment of alleles in genetic linkage analysis. Allele length can be precisely resolved to a single base by analysis of PCR products in polyacrylamide sequencing gels. The primers were designed for the nested PCR protocol in which a fragment of about 105 bp was finally generated. With a known sequence reaction ladder as the size marker, the sizes of the amplified fragments were determined accurately. The results were confirmed by DNA sequencing.

20

Deletion analysis of the human tissue kallikrein gene promoter. To determine the sequence requirements for the human tissue kallikrein gene promoter and the contribution of the polymorphic region to its transcriptional activity, a series of deletion constructs were created with 5' variable ends and a common 3' end at residue +10. The ability of the respective fragments to direct transcription of the luciferase reporter gene was analyzed by transient transfection assay in human embryonic kidney 293 cells. The construct pSV- β -galactoside (pCH110) was co-transfected with the luciferase reporter constructs to provide an internal control for transfection efficiency in each experiment. The resulting luciferase activity was normalized with β -galactosidase activity. The

normalized luciferase activities observed with difference deletions were compared to that of the -940/+10 (B) construct. Deletion of about 80% of the 5' flanking sequence (from -940 to -166) had little effect on the promoter activity (Figure 1). Further deletion to -98 caused a dramatic reduction of promoter activity by more than five-fold.

5 The control constructs with the promoter sequence in the anti-sense orientation showed very low promoter activities. These results indicate that the regulatory elements are present within the -166/-144 and -144/-98 regions, which are important as basal tissue kallikrein gene promoters. The region -166/-144 contributes to repression while the region -144/-98 contributes to stimulation of promoter activity. These results indicate a

10 functional role for the polymorphic region in regulating transcriptional activity.

Promoter activity analysis of various alleles. To determine the effect of this polymorphism on the transcriptional activity of the human tissue kallikrein gene promoter, nine -940/+10 deletion constructs, each containing one allele, were created.

15 Transient transfection assays were conducted to test the promoter activity of each allelic variant. Their promoter activities were tested in human 293 (embryonic kidney) cells (ATCC accession No. CRL 1573). Allele H exhibited only 10-20% promoter activity compared with other alleles and allele D showed less than 50% promoter activity (Figure 2). The other alleles have similar promoter activities. The plasmid

20 -940/+10 allele B in the antisense orientation and pGL2-Basic were also tested in this cell line as the controls. In these controls, the vector pGL2-Basic had 19% promoter activity and the construct +10/-940 B had only 1% activity.

Assay for correlating human tissue kallikrein promoter polymorphisms with

25 *increased or reduced risk of developing essential hypertension.* This large scale study consists of collecting urine and DNA samples for 332 individuals belonging to 60 families. Blood pressure data are also collected for each individual. Urine tissue kallikrein levels are quantified according to standard protocols known in the art. The promoter region of the individual's's tissue kallikrein gene will be sequenced to

identify the particular polymorphism (allele) in this region. A correlation will be made regarding an individual's genotype, urinary tissue kallikrein level and blood pressure, as analyzed using a two-tailed t test. The correlation can be extended further to include measurement of the amount of promoter activity demonstrated by a particular allele
5 according to the molecular biology protocols described herein.

Because applicants show that the allele in the tissue kallikrein gene promoter is correlated with blood pressure status, direct analysis and comparison of an individual's genotype and phenotype can be used to identify alleles correlated with increased or
10 decreased risk of developing essential hypertension. As a result of this correlation, it will be possible to predict an individual's risk of developing essential hypertension (phenotype) on the basis of the individual's genotype or amount of tissue kallikrein promoter activity, allowing for early intervention in individuals determined to be at risk and the prevention of the onset of essential hypertension and subsequent organ damage.
15

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

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Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Table 1. DNA sequence of alleles in the polymorphic region of the human tissue kallikrein gene

Alleles	Sequence	Length
A	5'-GGAGGGGGGGGGGGGC-3' (SEQ ID NO:14)	14
B	5'-GGAGGCGGGGGGGGC-3' (SEQ ID NO:15)	14
C	5'-GGGGGGGGGGGGGGGC-3' (SEQ ID NO:16)	14
D	5'-GGGGCGGGGGGGGGGC-3' (SEQ ID NO:17)	14
E	5'-GGAGGGCGGGGGGGGC-3' (SEQ ID NO:18)	14
F	5'-GGAGCGGGGGGGGGGC-3' (SEQ ID NO:19)	15
G	5'-GGAGCGGGGGGGGGGC-3' (SEQ ID NO:24)	14
H	5'-GGAGGGGGGGGGGGGGGC-3' (SEQ ID NO:20)	15
I	5'-GGAGGGGGGGGGGGGC-3' (SEQ ID NO:21)	13
J	5'-GGAGGGGGGGGGGC-3' (SEQ ID NO:25)	12
K	5'-GGAGGGGGGGGGGGGGGC-3' (SEQ ID NO:22)	16
M	5'-GGAGGGGGGGGGGGGGGGGC-3' (SEQ ID NO:26)	17
P	5'-GGAGGGCGGGGGGC-3' (SEQ ID NO:23)	13
R	5'-GGAGGGGGGGGGGGCGGC-3' (SEQ ID NO:27)	16

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Table 2. Allelic frequencies in the polymorphic region of the human tissue kallikrein gene

Alleles	Caucasians (57 subjects)		African-Americans (33 subjects)		Asians (18 subjects)	
	Numbers Observed	Allelic Frequency	Numbers Observed	Allelic Frequency	Numbers Observed	Allelic Frequency
A	48	0.421	13	0.197	22	.0611
B	33	0.290	12	0.182	4	0.111
C	0	0.000	4	0.061	0	0.000
D	0	0.000	4	0.061	0	0.000
E	1	0.009	4	0.061	0	0.000
F	0	0.000	1	0.015	0	0.000
H	16	0.140	6	0.091	9	0.250
I	2	0.018	12	0.182	0	0.000
K	14	0.123	9	0.136	1	0.028
P	0	0.000	1	0.015	0	0.000

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Table 3. Observed and expected numbers of genotypes in the polymorphic region among different populations, assuming Hardy-Weinberg equilibrium

Genotypes	Caucasians		African-Americans		Asians	
	(57 subjects)		(33 subjects)		(18 subjects)	
	Observed Numbers	Expected Numbers	Observed Numbers	Expected Numbers	Observed Numbers	Expected Numbers
Homozygote	12	16.90	6	4.73	7	8.08
Heterozygote	45	40.1	27	28.27	11	9.92
χ^2	2.0187		0.3981		0.2619	

SEQUENCE LISTING

(1) GENERAL INFORMATION

- (i) APPLICANT: MEDICAL UNIVERSITY OF SOUTH CAROLINA
171 Ashley Avenue
Charleston, South Carolina 29425
- CHAO, LEE
CHAO, JULIE
- (ii) TITLE OF THE INVENTION: METHODS AND COMPOSITIONS FOR
CORRELATING TISSUE KALLIKREIN GENE PROMOTER POLYMORPHISMS WITH
ESSENTIAL HYPERTENSION
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: NEEDLE & ROSENBERG, P.C.
(B) STREET: Suite 1200, 127 Peachtree Street, NE
(C) CITY: Atlanta
(D) STATE: GA
(E) COUNTRY: USA
(F) ZIP: 30303
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/856,141
(B) FILING DATE: 14-May-1997
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Miller, Mary L
(B) REGISTRATION NUMBER: 39,303
(C) REFERENCE/DOCKET NUMBER: 19113.0045/P
- (ix) TELECOMMUNICATION INFORMATION:
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(C) TELEX:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CTGCAGGAAT CTAGTTC

17

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGAACCAGG AACCACAT

18

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATCTGGAGGC ACTAGGA

17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCTAGTGCC TCCAGAT

17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTACCAGGGT CACTGAT

17

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATCAGTGACC CTGGTAC

17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATGCGTCC AGCGTGATCC

20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCCCTTATC CCCTCAGAGC

20

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTCTGGGGA TCGGAGGG

18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTGGGGATC GGAGGC

16

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTGATCCAGG GCCTGCA

17

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTTCCCCTGT TGCCTGG

17

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGAGGAACT GGGGAAC

17

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGAGGGGGGG GGGC

14

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGAGGCGGGG GGGC

14

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGGGGGGGG GGGC

14

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(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGGCGGGGG GGGC

14

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGAGGGCGGG GGGC

14

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGAGCGGGGG GGGGC

15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGAGGGGGGG GGGGC

15

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(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGAGGGGGGG GGC

13

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGAGGGGGGG GGGGGC

16

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGAGGGCGGG GGC

13

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGAGCGGGGG GGGC

14

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGAGGGGGGG GC

12

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGAGGGGGGG GGGGGGC

17

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGAGGGGGGG GGCGGC

16

What is claimed is:

1. A method for identifying a human subject as having an increased risk of developing essential hypertension, comprising determining the presence in the subject of an allele in the promoter region of the subject's tissue kallikrein gene correlated with an increased risk of developing essential hypertension, the presence of the allele identifying the subject as having an increased risk of developing essential hypertension.

2. The method of claim 1, wherein the allele comprises the nucleotide sequence of SEQ ID NO:17 (allele D).

3. The method of claim 1, wherein the allele comprises the nucleotide sequence of SEQ ID NO:20 (allele H).

4. A method for identifying a human subject as having an increased risk of developing essential hypertension, comprising:

a) correlating the presence of a nucleic acid sequence at nucleotides -166 through -98 in a human tissue kallikrein gene with an increased risk of developing essential hypertension; and

b) determining the nucleic acid sequence of nucleotides -166 through -98 of the subject's tissue kallikrein gene, whereby a subject having a nucleic acid sequence at nucleotides -166 through -98 in the tissue kallikrein gene correlated with an increased risk of developing essential hypertension is identified as having an increased risk of developing essential hypertension.

5. The method of claim 4, wherein the nucleic acid sequence correlated with increased risk of developing essential hypertension comprises the nucleotide sequence of SEQ ID NO:17 (Allele D).

6. The method of claim 4, wherein the nucleic acid sequence correlated with increased risk of developing essential hypertension comprises the nucleotide sequence of SEQ ID NO:20 (Allele H).

7. A method of identifying an allele in the promoter region of the human tissue kallikrein gene correlated with an increased risk of developing essential hypertension comprising:

a) determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the human tissue kallikrein gene from a subject; and

b) correlating the presence of the nucleic acid sequence of step (a) with the presence of essential hypertension in the subject, whereby the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene identifies an allele correlated with an increased risk of developing essential hypertension.

8. A method of identifying an allele in the promoter region of a human tissue kallikrein gene correlated with an increased risk of developing essential hypertension comprising:

a) determining the nucleic acid sequence of the nucleotides at positions -166 through -98 in the promoter region of the human tissue kallikrein gene;

b) cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene into an expression vector having a reporter gene;

c) expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and

d) determining the amount of reporter gene product produced, an amount of reporter gene product less than the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) or a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the

nucleotides at position -166 through -98 of the human tissue kallikrein gene as an allele correlated with an increased risk of developing essential hypertension.

9. A method for identifying a human subject as having an increased risk of developing essential hypertension comprising:

b) cloning the nucleic acid sequence encoding the promoter region of the human tissue kallikrein gene of the subject into an expression vector having a reporter gene;

c) expressing the reporter gene to produce a gene product under the direction of the promoter region of the human tissue kallikrein gene; and

d) determining the amount of reporter gene product produced, an amount of reporter gene product less than of the amount of reporter gene product produced under the direction of a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:14 (allele A) and a tissue kallikrein gene promoter containing the nucleotide sequence of SEQ ID NO:15 (allele B) identifying a nucleic acid sequence of the nucleotides at position -166 through -98 of the human tissue kallikrein gene as an allele correlated with an increased risk of developing essential hypertension.

10. A method for identifying an allele in the promoter region of the human tissue kallikrein gene, comprising determining the nucleic acid sequence of the nucleotides at positions -166 through -98.

11. An isolated nucleic acid consisting of the promoter region of the human tissue kallikrein gene between positions -1 to -940.

12. An isolated nucleic acid of up to about 940 nucleotides in length comprising the nucleic acid sequence of SEQ ID NO: 9.

13. An isolated nucleic acid of up to about 940 nucleotides in length comprising the nucleic acid of SEQ ID NO: 10.

14. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO: 14 (allele A).

15. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:15 (allele B).

16. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:16 (allele C).

17. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:17 (allele D).

18. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:18 (allele E).

19. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:19 (allele F).

20. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:20 (allele H).

21. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:21 (allele I).

22. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:22 (allele K).

23. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:23 (allele P).

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24. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:24 (allele G).

25. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:25 (allele J).

26. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:26 (allele M).

27. An isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO:27 (allele R).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/09831

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 297 913 A (AMGEN INC) 4 January 1989 see table V page 13	13
X	& DATABASE EMPATENT embl AC:I08086, LIN & LU: "Production of kallikrein" see abstract	13
A	WO 87 02709 A (BIOTECH RES PARTNERS LTD) 7 May 1987 see the whole document	1-27

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

24 August 1998

Date of mailing of the international search report

04/09/1998

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/09831

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PRAVENEC M. ET AL.,: "Cosegregation of blood pressure with a kallikrein gene family polymorphism" HYPERTENSION, vol. 17, - February 1991 pages 242-246, XP002075077 see the whole document ----	1-27
A	WOODLEY-MILLER C. ET AL.,: "Restriction fragment length polymorphisms mapped in spontaneously hypertensive rats using kallikrein probes" J. OF HYPERTENSION, vol. 7, - 1989 pages 865-871, XP002075280 see the whole document ----	1-27
P,X	SONG Q. ET AL.,: "DNA polymorphisms in the 5'-flanking region of the human tissue kallikrein gene" HUM. GENET., vol. 99, - June 1997 pages 727-734, XP002075078 see the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

I. International Application No

PCT/US 98/09831

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0297913 A	04-01-1989	AT 118547 T	15-03-1995
		AU 2075588 A	30-01-1989
		DE 3853023 D	23-03-1995
		DE 3853023 T	08-06-1995
		DK 91389 A	01-05-1989
		ES 2068201 T	16-04-1995
		FI 890889 A	24-02-1989
		GR 3015881 T	31-07-1995
		JP 1503679 T	14-12-1989
		PT 87887 A, B	31-05-1989
		WO 8900192 A	12-01-1989
WO 8702709 A	07-05-1987	US 4861708 A	29-08-1989
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		DK 321987 A	24-06-1987
		EP 0234095 A	02-09-1987
		JP 63501900 T	28-07-1988

